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OBSERVATIONS ON POLARITY IN THE SLIME MOLD DICTYOSTELIUM DISCOIDEUM

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In previous work done on the amoeboid slime mold, *Dictyostelium discoideum*, there was good evidence that a chemical substance called acrasin is involved in all its morphogenetic stages. The substance was first demonstrated as responsible for the bringing together of the myxamoebae by chemotaxis in the aggregation stage (Bonner, 1947). Then it was shown that the same substance was continually emitted, in varying amounts depending on the stage of development and the part of the cell mass or pseudoplasmodium, until the final, mature spore-bearing fruiting body was formed (Bonner, 1949). During these later migration and final culmination stages (Raper, 1935, 1940a; Bonner, 1944, for details of the life history) it was shown that there was a high correlation between the amount of acrasin produced by a region and the differentiation of that region into spore or stalk cells (Bonner and Slifkin, 1949).

There are, in the morphogenesis of this slime mold, certain manifestations that can be grouped under the general term polarity, a word which implies a directional quality, a headness and tailness, a symmetry to the developing organism. The question before us (and this paper by no means answers it, but only clarifies the issue to some extent) is what precisely is the relation between polarity and the acrasin mechanism. The mere fact that it is always the anterior end of the migrating pseudoplasmodium that becomes the high region of acrasin emission shows that the two processes are interconnected, but we know little more than this. Polarity is so fundamental and yet so mysterious an aspect of development that any description of some of its activities, which is all I offer here, might ultimately help in guiding us to the real answers.

Observations and Experiments

Vegetative myxamoebae. Polarity in any amoeba presents a special problem, for in most organisms polarity refers to a fixity of direction which is expressed by the fixity of the parts, and in an amoeba there is little fixity of parts. An amoeba may crawl in one direction for a great period of time, but in doing so practically every particle of its substance will pass a moment, however fleeting, at its anterior end, or at any other point. It is true that the nucleus will remain somewhere in

[1] This work was carried out with the help of a grant from the American Cancer Society.

the very general vicinity of the center and also there are occasional evidences of a somewhat more permanent foot or posterior region, but even these phenomena show great variation from moment to moment. And some years ago Jennings (1904) showed, by watching small particles that adhere to the outside surface of an ordinary amoeba, that the surface membrane itself moves in relation to the whole amoeba, by a sort of rolling action.

At any one instant vegetative myxamoebae have a polar appearance, for although their contour is irregular and amorphous, one section of their border (approximately ¼ to ½ of the circumference) is hyaline, while the rest is granular. This is well illustrated in some drawings by Raper (1941). But, just as we have seen in the case of the true amoebae, this polarity is ephemeral.

Aggregating myxamoebae. The myxamoebae themselves, when they commence aggregating, are more elongated than during the vegetative stage, stretching in length to 5 or 6 times their diameter (Bonner, 1947, for photographs of aggregating myxamoebae). But nevertheless, the problems which were mentioned for the vegetative myxamoebae concerning the constant mixing and reshuffling of the internal parts, apply equally well for the elongate aggregating myxamoebae. There is a curious cap on the nucleus which can be seen in stained preparations and with the phase contrast microscope, but even it is not consistently oriented in respect to the aggregation center but is pointed about 50 per cent of the time towards the center and 50 per cent away.

The nearest phenomenon to fixity of parts that is found in aggregating myxamoebae is that the nucleus usually lies in the anterior half of the cell. This is clearly seen in living myxamoebae with an oil immersion phase contrast microscope.[2] The nucleus does, of course, jostle about severely during movement, but its position is most often in the second quarter of the cell from the anterior end. The anterior-most quarter is hyaline and lacks any visible inclusions whatsoever.

Since the aggregating myxamoebae are presumably oriented in their movement by a diffusion gradient of acrasin, it seemed of interest to see what effect suddenly reversing the acrasin gradient might have on them. This was first done in repeated experiments on isolated aggregating myxamoebae and on myxamoebae in thin streams. The center towards which they were moving was simply removed and placed directly behind them. These were done in under-water preparations, using standard solution (Bonner, 1947). Invariably one of two things happened. Occasionally the myxamoebae balled up and after considerable kneading of the surface of the sphere it would send out a pseudopod in the direction of the center at its new location. More frequently the myxamoeba would make a "U" turn without ever losing its elongate shape (see Fig. 1, A).

In another type of experiment a section of a stream of incoming myxamoebae was removed, and reversed. The section, usually about 200 μ long, was cut with a glass needle and turned 180°. If the piece was kept separate from the remaining stream it first contracted and then the individual myxamoebae separated from the

[2] The technique used here was the "thin film of water technique" given in detail by Bonner (1947). The only modification was that the coverslip containing the myxamoebae was placed over a van Tieghem cell filled with light Parke-Davis mineral oil. This greatly enhanced the optical conditions for the phase microscope. Bausch and Lomb phase contrast equipment was used: a 97 × objective, and a long working distance condenser.

piece and moved independently towards the new center (Fig. 1, B). No difference in behavior could be detected between cases where the section was cut and reversed, or merely cut. If the reversed piece was touched to the remaining stump of the stream connected to the center, then they adhered fast to one another. Almost immediately after the operation the reversed section became short and thick and it could be seen that this was because each myxamoeba had become rounded. This rounded appearance of the myxamoebae first reverted to the normal elongated form at the central end of the reversed section as though the myxamoebae had been

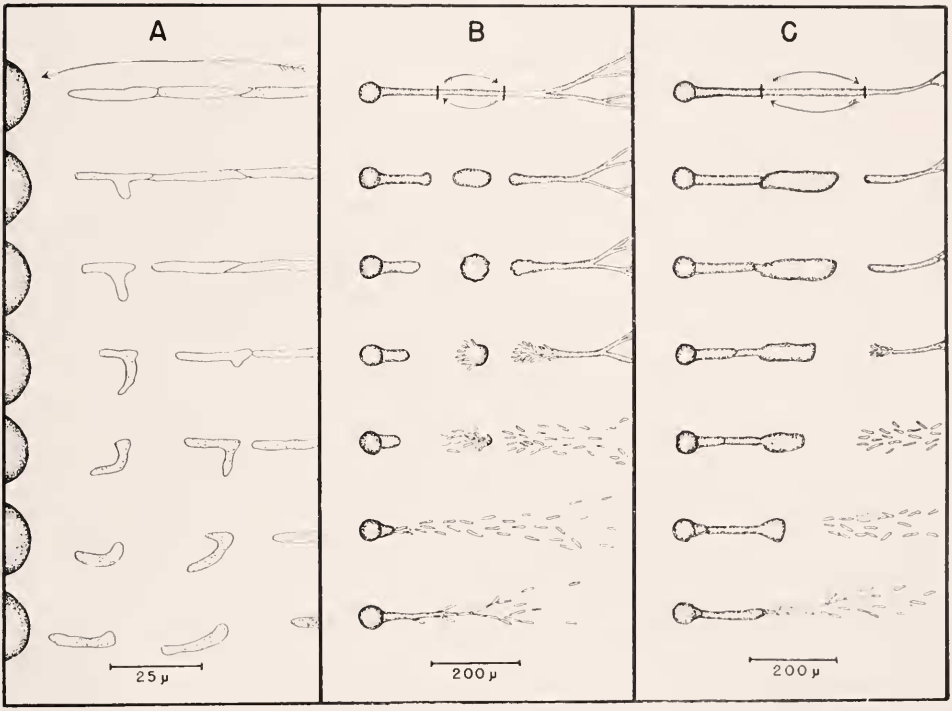

FIGURE 1. Diagram illustrating the experimental reversal of an acrasin gradient during aggregation. A. In the top diagram a center has been removed from in front of the chain of myxamoebae and placed behind. The succeeding drawings show the resulting changes at intervals of 2 min. B. A section of the stream has been reversed (top) and the resulting changes are shown at 4 min. intervals. C. The same as B except that the reversed piece was attached to the proximal stump of the stream.

pulled into their normal elongate shape. In each attempt this passage from the rounded to the elongated form progressed distally until a stream normal in appearance was achieved (Fig. 1, C).

One last curious phenomenon concerning aggregation should be mentioned here and that is the observation of Arndt on aggregation centers that form whirlpools (Arndt, 1937; Raper, 1941). Instead of the amoebae coming directly to a point, they come in at an angle (exactly the way water enters a whirlpool) and the cell mass is not solid, but ring-shaped with a hollow center. Using the under-water

preparations (Bonner, 1947) whirlpool centers often appear and if one watches the ring one can see that the cells follow each other round and round, the ring constantly decreasing in diameter, until the hole in the center disappears; forming finally a solid cell mass. But the important point here is that the cells in the ring move in a polar fashion, even though there is no dominant point, but only a continuous ring.

Migrating pseudoplasmodia. In its external form the migrating pseudoplasmodium is obviously and clearly polar. The anterior end is radially symmetrical and tapers down to a characteristic "bullet-nose," while the posterior end is broad and bilaterally symmetrical, tapering down to a small flat tail. Furthermore the motion shows a fixed polarity and a slime track is deposited posteriorly, that is, it comes off the posterior end.

Previously (Bonner, 1944) serial sections were made of migrating pseudoplasmodia and it was noted that ordinarily there was no marked orientation of the myxamoebae within the pseudoplasmodium except in the anterior portion of late migrating pseudoplasmodia. There the long axis of the cells was at right angles to the long axis of the whole pseudoplasmodium. Upon careful re-examination of the slides it can be seen that the posterior cells occasionally show slight orientation in the antero-posterior direction, most frequently in the cells near the slime sheath. Perpendicular orientation of the anterior cells was also observed in the pre-stalk cells of the tip of the culminating pseudoplasmodium.

A series of studies were made in which the pseudoplasmodia were made to crawl on the underneath side of coverslips, and the cells were observed with the high powers of the microscope (43 × and 97 × objectives). Invariably all the cells that could be seen were actively involved in amoeboid motion continually giving off pseudopodia. With these preparations it was impossible to see the perpendicularly oriented cells in the anterior end, but it was found that if a culminating pseudoplasmodium was placed in a small drop of mineral oil between a slide and a coverslip and the preparation was observed with the phase contrast microscope (43 × objective) the outlines of the cells could be seen.[3] Motion pictures of this were taken with a 16 mm. movie camera at 500 × normal speed, and these showed an even, smooth forward movement towards the tip of all the cells, despite their perpendicular orientation relative to their axis of motion.

It is difficult to reconcile the fact that the long axes of the constituents cells do not always lie in the same direction of the long axis of the whole migrating pseudoplasmodium. Another problem is that since the cells of migrating pseudoplasmodia continue to be pseudopodial, presumably they too (as did the isolated myxamoebae) must have their parts in a continual flux. The puzzling question here is how it is that with each cell in constant turmoil and showing little or no orientation with respect to its movement, that the whole migrating pseudoplasmodium shows polarity of shape and of movement. In an effort to analyze this problem further, experiments attempting to reverse sections of migrating pseudoplasmodia were made.

The first experiments involving reversing sections of migrating pseudoplasmodia

[3] A different species *Polysphondylium violaceum* was used here as they were somewhat easier to handle for this purpose. No difference in respect to the orientation could be observed between it and *Dictyostelium discoideum*.

were done by Raper (1940b). He found that if he cut off a posterior section and replaced it after reversing it 180°, then invariably the pieces crawled away from one another; never fusing and always retaining their original direction of movement. This experiment is easy to perform and I have obtained the same results with many tries.

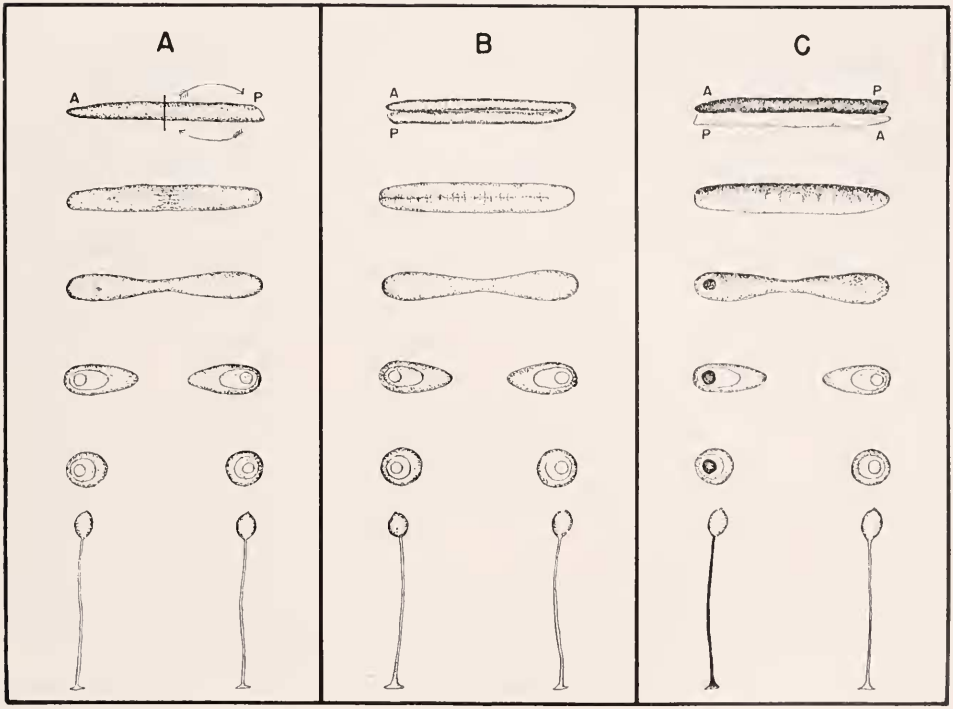

FIGURE 2. Diagram showing the experimental reversal of parts of the migrating pseudoplasmodium. A. The posterior half of a migrating pseudoplasmodium has been reversed and fused carefully onto the anterior half. The changes resulting are given at 15 min. intervals except for the final fruiting bodies drawn at the bottom which have appeared after an interval of 10 hrs. (For convenience the mature fruiting bodies are represented disproportionately small in this figure.) B. A migrating pseudoplasmodium is folded onto itself and the resulting changes are recorded as described for A. C. Two migrating pseudoplasmodia oriented in opposite directions and the upper one stained with nile blue sulphate are carefully fused and the changes are recorded as described for A. Note that the blue and colorless cells intermingle except at the anterior tips.

The point in question here is whether or not, provided the conditions set up in the experiment are just right, a section can be made to reverse if it is under the influence of a high source of acrasin in the close vicinity. One of the possible difficulties encountered in the type of experiment Raper used might be that the slime sheath crowded or herded the cells together, thereby physically preventing them from responding to acrasin. In order to avoid this after a section was reversed, it was gently stirred with an extremely fine glass needle so as to rupture the slime sheath, and carefully fused in this way to the unreversed section. Again the results

were the same, for despite this disruption of the sheath the original polarity of each piece was retained bearing no relation to the overall acrasin emission gradients (Fig. 2, A).

A number of variations of these experiments were undertaken and in each case the object was to cause a reversal of the polarity of a part of the pseudoplasmodium.

In one variation, pseudoplasmodia were folded back so that the two halves touched and the anterior and posterior ends were just opposite each other. The halves were then fused together by passing a fine glass needle back and forth through them. This folded pseudoplasmodium would soon divide into two, forming one center at the "anterior-posterior" end, and another at the "middle" end. These centers were of approximately equal size indicating that the cells retained their original direction of motion, rather than being influenced by the presumably dominant tip region (Fig. 2, B).

In another group of experiments, whole migrating pseudoplasmodia were placed along side each other so that the anterior end of one would lie along side the posterior end of the other. Again they were fused with a needle and again two pseudoplasmodia about equal in size formed at each end of the mass of cells. In some cases the anterior $\frac{1}{10}$ of one of the pseudoplasmodia was removed, but the result of the experiment was the same.[4]

These same experiments were repeated but one of the pseudoplasmodia was stained with nile blue sulphate. Then, after fusion, it was possible to follow the cells of the two pseudoplasmodia. Invariably the two new centers contained both blue and colorless cells, although the center which arose at the site of the anterior end of the blue pseudoplasmodium was predominantly blue and the converse was true of the other center (Fig. 2, C). So we see that although the original polarities of the two pseudoplasmodia were maintained there was considerable mixing of the cells. It was, of course, impossible to ascertain to what extent this was due to the reversing of cells by literally turning them about with the needle, but conceivably this could completely account for the phenomenon.

One final experiment on the migrating pseudoplasmodia should be mentioned, in which pseudoplasmodia were coiled in a ring so that their anterior ends just touched their posterior ends. The two ends were then fused with a needle. After a period of 15 minutes or so, a separation line would appear at its original place and the anterior end would move off in another direction. This would occur even in cases where there was known to be an even acrasin emission the length of the pseudoplasmodium, and in cases where the whole ring was gently stirred with a needle. Apparently the difference between the anterior and posterior ends is sufficient so that their individuality is retained.

Shaking experiments. Using the technique of obtaining myxamoebae for under water preparations (Bonner, 1947), washed myxamoebae in standard solution were placed in 125 cc. Erlenmeyer flasks. The flasks were set on a shaking machine which gently rocked them back and forth. Even though the myxamoebae were at their vegetative stage, after about 5 to 10 minutes of shaking they clumped into

[4] It should be mentioned that in all these experiments, if the disrupting with the needle is sufficiently great, many small centers will arise and therefore care was taken to apply the needle gently.

spherical balls of cells. With continuous shaking for 12 hours these balls of cells became quite polar in appearance, for one end (presumably anterior) was relatively pointed, the other end (posterior) exuded slime sheath material (Fig. 3). It is

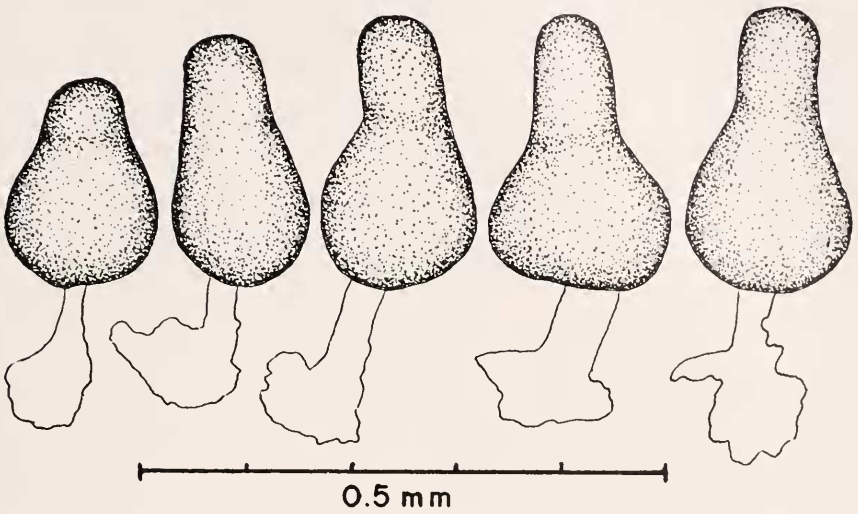

FIGURE 3. Camera lucida drawings of a number of the regular cell masses appearing after 12 hrs. of shaking. Note the slime sheath material issuing from one end. These drawings are of cell masses from one flask and are an example of the most regular forms obtained.

quite remarkable that the over-all polarity becomes evident in a spherical ball of cells under constant agitation. These polar masses were placed among random myxamoebae which oriented towards the mass, indicating that the mass was actively emitting acrasin.

DISCUSSION

One fact that clearly emerges from the above observations is that the over-all, total morphological polarity shows a consistent relation with the polarity of movement, but the smaller constituent parts show little or no relation. In aggregation the external contour of the myxamoebae reflects its polar movement, and also in migration the direction of movement is reflected by the shape of the whole pseudoplasmodium. But in aggregation the parts of the myxamoebae show no such relation (with the possible exception of the position of the nucleus) and in migration, not only the internal parts of the myxamoebae, but also the myxamoebae themselves do not orient with respect to the polarity of the migrating pseudoplasmodium.

It is clear that however the polarity arises in the migrating pseudoplasmodium, it is not necessarily dependent on the polarity acquired during aggregation, for in the shaking experiments it was shown that the polarity could arise in a ball of cells thrown together quickly. The experiments in which the migrating pseudoplasmodia were placed in a ring show that not only is the direction of the movement important, but that there are discreet (although unknown) differences between the

anterior and the posterior end, for these will not fuse but separate from one another after artificial fusion.

In aggregation the gradient of acrasin is capable of governing the polarity, for the myxamoebae invariably orient towards a high concentration of acrasin (Bonner, 1947). However, this is probably not the only factor involved for the adhesiveness of the myxamoebae played a part in the experiment where a section of reversed aggregation stream was attached to another stream and literally pulled into the center (Fig. 1, C).

The best evidence that an external acrasin gradient is not necessarily required for orienting the aggregating myxamoebae comes from the whirlpool configuration, for in that case there is a line of myxamoebae moving in a polar fashion, yet presumably they are all in the same external concentration of acrasin. Even if there were a high point of acrasin production at some region on the ring, then the argument that the acrasin gradient is not orienting the myxamoebae would still hold: for some of the myxamoebae in the ring must be moving away from the region of high acrasin concentration.

In the migrating pseudoplasmodium there is also abundant evidence that an over-all external acrasin gradient does not necessarily govern the polar movement. In the first place, even though the young migrating pseudoplasmodia move about with over-all polarity, they do not possess a gradient of acrasin emission but emit the acrasin equally in all parts (Bonner and Slifkin, 1949). Also, in the experiments in which portions of the pseudoplasmodia were reversed, in no case did the movement of the sections reverse and go towards a high acrasin producing tip (in older pseudoplasmodia the tip produces more acrasin than the remaining portion, Bonner, 1949).

We have then a good demonstration that an over-all acrasin gradient does not account for polar movement or polar shape. There is, unfortunately, no way as yet of determining the distribution of acrasin inside the cell mass, for it is not at all certain that the over-all emission of acrasin reflects its internal distribution at all.

Summary

Experiments designed to give some insight into the nature of polarity were performed on the amoeboid slime mold *Dictyostelium discoideum*. The separate myxamoebae which stream together to form aggregations of myxamoebae show an external polarity and the resulting cell mass has a clear over-all antero-posteriority. In both cases it was shown that the internal parts do not reflect the external polarity. It is known from previous experiments that the aggregating myxamoebae orient in a concentration gradient of a chemical substance tentatively called acrasin, and that acrasin is found in later stages of development. By reversing the gradient of acrasin, separate myxamoebae did not back up but re-oriented towards the point of high concentration. However, it was shown by various experiments that the external acrasin gradient cannot always determine the direction of the polarity, for in the cell masses the polar movement occurs when there is no external gradient of acrasin, or in some cases away from the region of high acrasin emission. It was also shown that polarity can arise in spherical cell masses undergoing gentle shaking in liquid media.

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